FPR onto Ni^{2+}-NTA silica particles was demonstrated by the depletion of receptor from FPR extracts. The experiments were performed with 1nM fMLFK-FITC, $1.5x10^7$ cell equivalents/ml of membrane and 20 mg silica particles/ml. The spectroscopic analysis used the antibody to fluorescein to examine ligand binding. The binding curves are depicted from top to bottom: receptors present on silica particles, receptors present in the membrane extract, receptors present in the supernatant after silica particles have been removed from the extract, control sample in which a blocking peptide (10^{-5} M tboc-phe-leu-phe-leu-phe) (SEQ ID NO. 1) inhibits the specific binding. In the presence of the particles, receptors were quantitatively sedimented out of bulk phase. Binding of ligand to the particle-bound receptors resulted in an increased ligand binding signal (due to slower ligand dissociation and a higher binding affinity). Figure 3b compares the ligand–receptor dissociation characteristics. Dissociation rates are determined from Fig. 3b by subtracting the non-specific binding in the blocked control from the specific binding and replotting the data on a semi-log scale. From top to bottom the curves are: the ligand dissociation from receptors in the membrane extract in the presence of the silica particles; the ligand dissociation in the particulate fraction of the extract after pelleting by centrifugation and resuspension; the ligand dissociation from solubilized receptors; the ligand dissociation from the supernatant of particles and solubilized receptors.

B2 concl.

Please delete the paragraph at page 15, line 10 to page 16, line 8 and substitute therefor the following paragraph:

Experiments to assess the quantitative affinity-coupling of soluble C-his FPR to Ni^{2+}-NTA silica particles and the relative affinity of the receptor on the substrate for ligand were performed using a FACScan flow cytometer (Becton Dickinson Immunocytometry, San Jose, CA). Ten thousand events were analyzed per sample, using a threshold on forward angle light scatter and forward angle versus 90^o light scatter dot plot gating to resolve the primary population of silica particles. Data was

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collected from FL1 (FITC fluorescence) in log mode with no spectral compensation. Figs. 4a and 4b show light scatter characteristics of silica particles by flow cytometry in the presence of specific (a) and non-specific (b) ligand binding. Fluorescence histograms of specific (Fig. 4c) and non-specific (Fig. 4d) ligand binding were compared to (Fig. 4e) fluorescence histograms of quantitative bead standards. The flow cytometric dot plot of SSC vs FSC shows that the particles are heterogeneous (Figs. 4a and b). However, the FL1 histogram data shows that there is specific ligand binding (Fig. 4c), compared to the non-specific binding signal obtained when antagonist is present (Fig. 4d). An estimate of the number of receptors displayed per particle was made using calibration standards for fluorescein labeled ligands (Fig. 4e). The average number of fluorescein equivalents per particle was about 1.5 million, similar to the highest standard. In order to convert flow cytometer data to ligand binding measurements, several additional factors must be taken into account: the relative fluorescence of free fluorescein compared to conjugated FITC (85%) and the quenching upon binding to the receptor. The number of receptors occupied at particle saturation is therefore estimated to be ~ 2 million. Taking into account the K_d and the ligand concentration, as described in Fig. 5 below, the total number of binding sites per article is on the order of 3 million. Under optimal conditions, a fluorescent ligand signal to background ratio of at least 30:1 can be obtained (Fig.4f). The optimal signal is obtained by varying the input of the receptor at fixed particle density with the signal saturation at an input of receptor above 10-15 million cell equivalent/ml. Samples were prepared and analyzed at 4°C. The five bead populations represented 0, 48,900, 87,400, 552,000, and 1,510,000 fluorescein equivalents. Experiments were performed with 10 nM fMLFK-FITC, 10 mg/ml silica particles, and $1.5x10^7$ CEQ/ml. The blocking peptide t-boc-phe-leu-phe-leu-phe (SEQ ID NO. 1) was used at 10^{-5} M. Controls included silica particles with no receptor in the presence or absence of *f*MLFKF-FITC and samples in which the binding of *f*MLFK-FITC was inhibited by preblocking with t-boc-phe-leu-phe-leu-phe (SEQ ID NO. 1) or F-met-leu-phe-phe-gly-gly-lys (SEQ ID NO. 2).

B3 cont.